**Effects of incorporation Spirulina platensis (Gomont, 1892) powder in wheat flour on chemical, microbial and sensory properties of pasta**

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Received: April 2017          Accepted: August 2017

**Abstract**

*Spirulina platensis* a blue-green microalgae with unique nutrient content and numerous nutritional and therapeutic effects are employed in enrichment of various food products. Information about fortified pasta with *S. platensis* microalgae is very low. *S. platensis* microalgae powder at a level (% W/W), 0.0, 0.25, 0.5, 0.75 and 1 %, was added in pasta formulation. Protein, iron, microbial and sensory properties of product were analyzed. Microbiological testing of the samples, was conducted according to the national standard methods. Sensory evaluation was conducted by 60 panelists using hedonic method. The results showed that different levels of microalgae *S. platensis* powder had a significant effect on chemical parameters of pasta (*p*<0.05). Also interaction of microalgae powder on microbial features of pasta was not significant (*p*>0.05). Sensory evaluation test indicated greater acceptability of pasta fortified with microalgae *S. platensis* powder at a concentration of 0.25%, as compared to the control sample. With the addition of 0.25% of *S. platensis* microalgae powder to pasta formulation, while reaching enriched product, nutritional value and sensory characteristics of pasta heals and a functional food will be available to the community.

**Keywords:** Pasta, Sensory evaluation, Enrichment, Microalgae, *Spirulina platensis*

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Introduction
Pasta products are simple types of semolina durum wheat (Durum) and water. Simple and easy formulation process of pasta has led it to be recognized as a relatively cheap food produce (Ghiassitarzi et al., 2012). Of wheat flour used to make pasta and pastry products, but durum wheat semolina is known as the best raw material throughout the world due to its favorable physicochemical properties in batter condition (Park and Kim, 1990). In the normal mode of plants are a good source of vitamins such as thiamin, riboflavin, and niacin tocopherol and minerals (iron and zinc), but, lots of these material disappears during the grinding process, because they are concentrated in the bran. There are major differences between the nutritional value of various pasta products by adding materials such as egg, spinach, tomatoes, vegetable protein concentrates, dairy products as well as vitamins and minerals. Consumption of these products is recommended due to the low level of sodium, fat and saturated fatty acids, and also cholesterol and high level of carbohydrate. However, macaroni products, like any other food source alone is unable to provide all the nutritional needs of humans (Pomeranz, 1988). In addition to the factors of production, the limited number of raw materials used to make pasta a perfect platform to evaluate the potential nutritional and technological adding raw material has higher nutritional value. Ingredients such as flavors, colors, vitamins, proteins, emulsifiers and in general materials with higher nutritional value to improve the color, taste, nutritional value, texture, and other sensory characteristics of the products used (Ghiassitarzi et al., 2012).

Spirulina platensis blue-green microalgae is well Known due to its unique nutritional quality. This microalgae contains high protein (60 to 70 percent dry weight), low-fat, high-amount of vitamins, especially vitamin B12, iron, phycocyanin pigments and essential fatty acid GLA, and could be a food with high nutritional value (Belay et al., 1993). As the World Health Organization (WHO) of Spirulina as the best food on earth has learned the US space agency (NASA) of the biomass of Spirulina in space travel as compressed food uses (Khan et al., 2005).

Numerous medical studies on the therapeutic effects of Spirulina, such as lowering blood cholesterol levels, protect against some cancers, prevention of cardiovascular diseases and increased resistance to the immune system has been carried out (Belay et al., 1993). So far, the powder S. platensis biomass to produce food products such as soups, sauces, snacks, drinks, sweets, chocolates, biscuits, bread, cakes and flour have been fortified (Yamaguchi, 1996; Gouveia et al., 2008). Also in Germany, a number of factories producing food products from microalgae and cyanobacteria in products such as yogurt and various drinks have used (Pulz and Gross, 2004). Micro-algae S. platensis is cheap and a rich source of protein, iron,
vitamin B12 and fatty acid, gamma linolenic acid (Kumudha et al., 2010). *Spirulina* benefits has been found from many years ago, because of the high levels of protein, vitamins, essential amino acids and essential fatty acids, and has been known that 60 to 70 percent of dry matter of *Spirulina* is protein and rich source of vitamins, especially B12 (which is usually found in animal tissues) and precursor of vitamin a (beta-carotene) and minerals, especially iron. Sheet and food additives used in aquaculture and poultry industry (Belay, 2002).

In a study in 2009, 550 malnourished children were fed with 10 gr. with *Spirulina* powder per day, without any adverse effects observed. Dozens of studies of human medicine so harmful effect of the supplement *Spirulina* is not provided. Food and Drug Administration (FDA) word GRAS (generally recognized as safe and healthy) as prepared in the two US companies donated *Spirulina* (Carlson, 2011). Kadam and Prabhasankar (2010) announced that the sensory evaluation of pasta samples showed that samples containing more than 10 percentage points higher admission Marine Wakame microalgae powder received by sensory evaluation. *Spirulina* also been observed with the use of pesticides preventing injuries affecting the heart, liver, kidneys, neurons, eye, ovary, DNA and testes (Belay and Gershwin 2007). Several studies have investigated the effect of adding different ratios of the different species of microalgae in bakery products was conducted. Powell and colleagues' study in 2011 by adding a homogeneous mixture of species *Chlorella* and *Scenedesmus* the combination of Ginger Bread, cakes and cookies spinach or green tea taste bitter taste is similar algae, and algae found in all foods dominance, color food that used to be changed (Powell et al., 1961). Danesi et al. (2010) showed that the protein can be used to enrich bakery products from micro-algae *S. platensis* use, without considerable changes in tissue expansion coefficient, the percentage composition and sensory acceptance of the product (Danesi et al., 2010). Sources indicate that information about micro-algae *S. platensis* powder enriched wheat flour for pasta production is very low. *S. platensis* unicellular algae, photosynthesizing, with spring-like filaments and belongs to the family of cyanobacteria that grows naturally in alkaline lakes and tropical America, Mexico, Asia, Central Africa (Belay, 2004). Pasta is so different processes including: kneading semolina with water, extrusion, pressing through the mold and finally drying is achieved. Various factors in the processes that affect the quality of the final product such as mixing time and speed spiral rotation, extrusion and mold temperature control, pressure adjustment, drying and ... (Ghiassitarzi et al., 2012). In the present study the possibility of wheat flour fortification at 0.0%, 0.25%, 0.5%, 0.75% and 1% w / w with microalgae *S. platensis* powder was investigated and the effect of varying the amount of microalgae powder used on features chemical,
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**Materials and methods**

**Raw materials**

Semolina flour with 26% wet gluten and 14% moisture content was used for pasta development.

*Spirulina* microalgae powder preparation

*Spirulina* microalgae powder was obtained from Parsiyan microalgae company (Guilan, Iran). The powder was packed in polypropylene bag with aluminum layer and was kept in cool, and dry conditions, away from sunlight until pasta processing.

**Sample preparation and production of pasta**

To prepare pasta enriched with *Spirulina* powder a processing standard formulation including semolina flour, gluten and water obtained from pasta company (Khoshnam food company, Isfahan, Iran) was used. Microalgae powder in five different stages and levels of 0.0 (control), 0.25, 0.5, 0.75 and 1 percent by weight of the composition of standard and experimental product was produced (Fradique et al., 2010). All samples prepared in the line of KHOSHNAM food company (Mak pasta). Short line (form) of the plant to build enterprise it (FAVA) Italy was used. Input Capacity pasta production line is 1500 kg of flour per hour.

First, the control sample consisting of semolina flour, water, gluten and beta-carotene were produced and then on the desired percentage, micro-algae *S. platensis* powder in 0% (control), 0.25%(T1), 0.5%(T2), 0.75%(T3) and 1%(T4) w/w (Table 1) to combine raw materials added. After mixing, the batter was passed by pressure from the mold and cut to the same size by the blade that was mounted on the mold outlet. Then, the pasta, were entered to pre drying. Finally, they transferred to the main dryer. The drying temperature is 77 °C. Pasta in this stage in 2 hours and 48 minutes were spent and the optimum moisture content of 12%.

**Chemical tests**

*S. platensis* micro-algae powder and pasta samples obtained in terms of the amount of iron based on AACC method 40-70.01, Micro-Kjeldahl based on protein content by a factor of 2.6 was measured using AACC method 46-12.01 (Batista et al., 2011). Water samples according to AACC Method 15A-44 and ash samples were measured according to the AACC method 08-01 (AACC, 2000).

**Microbiological tests**

The microbiological tests of the samples were performed with Iranian national standard No.2393. Tests include total count of microorganisms based on national standards 5272, 11166 *coliforms* based on national standard, based on the national standard 3-6806 coagulase positive *Staphylococcus aureus*, *Clostridium perfringens* according to standard procedures 2197, *Salmonella* according to standard procedures 1810 National
and mold based on national standards 2-10899, were studied (ISIRI, 2009).

Sensory evaluation test
Hedonic sensory evaluation was conducted using 5-point scale unspecialized by 60 panelists.
Sensory evaluation table is as follows:
1 = totally unacceptable
2 = slightly unacceptable
3 = neither unacceptable, nor acceptable
4 = less acceptable
5 = totally acceptable.

Sensory evaluators, after testing the samples, from 1 to 5 on the color, odor, taste, texture and overall acceptability scores were holder form of assessment forms for samples (Shogren et al., 2006).

Statistical analysis
Data were subjected by MINITAB software (versions 14, Minitab Inc, USA) and analysis of variance (one-way ANOVA) at 95% confidence level using Tukey multiple range test is done (Ozyurt et al., 2015).

Results
The proximate composition of microalgae S. platensis powder and flour and semolina. Results are shown in Table 1. Average chemical test pastes enriched with microalgae at 0.0% (control) 0.25%, 0.5%, 0.75% and 1% w / w is presented in Table 2.

Table 1: Proximate composition of micro-algae Spirulina platensis powder and semolina flour.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Semolina flour</th>
<th>Spirulina platensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein(gr per 100 gr)</td>
<td>9.8</td>
<td>57.47</td>
</tr>
<tr>
<td>Iron(mg per 100 g)</td>
<td>20960</td>
<td>125000</td>
</tr>
<tr>
<td>Ash(gr per 100 gr)</td>
<td>0.42</td>
<td>23.08</td>
</tr>
<tr>
<td>Moisture(gr per 100 gr)</td>
<td>14</td>
<td>7.46</td>
</tr>
</tbody>
</table>

Effects on protein
Statistical analysis showed that the protein content was no significant difference between cases and controls (p>0.05). Due to the fact that, according Tukey no significant differences were observed between samples, but based on the analysis of variance, respectively, of the fourth, third, first and second values showed more protein than the control sample.

Effects on iron
Based on the results of the statistical analysis of the iron index, there is a significant difference between the samples and controls (p<0.05) with control samples showed significant (Table 2).

Effects on ash
The results showed that in the final product produced with different percentages of the blank is a significant difference (p<0.05). In all phases of the study, S. platensis microalgae powder increases the amount of finished product ash (Table 2). This is due to high levels of minerals in S. platensis microalgae powder. The results showed that samples produced at percentages of 0.25 %, 0.5 % and 0.75% of Spirulina powder, more ash.
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Table 2: Chemical composition of pasta enriched with different levels of *Spirulina platensis*.

<table>
<thead>
<tr>
<th>Samples</th>
<th>T0</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (gr per 100 gr)</td>
<td>9.695±0.007&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.790±0.085&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.750±0.354&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.270±0.014&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.345±0.049&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Iron (mg per 100 g)</td>
<td>21.535±0.813&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.860±0.283&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.300±0.156&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.995±1.336&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.290±0.071&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ash (gr per 100 gr)</td>
<td>0.480±0.148&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.600±0.106&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.585±0.155&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.600±0.028&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.505±0.070&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Moisture (gr per 100 gr)</td>
<td>7.725±0.148&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.175±0.106&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.110±0.155&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.420±0.028&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.050±0.070&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Values in the same line followed by different letters indicate significant differences (p<0.05).

T0 (Treatment without microalgae powder (control)), T1 (Treatment with 0.25% microalgae powder), T2 (Treatment with 0.5% microalgae powder), T3 (Treatment with 0.75% microalgae powder), and T4 (Treatment with 1% microalgae powder).

*Spirulina* powder product containing 1% less ash than others listed percent, but was higher than the control sample.

**Effects on moisture**

The results showed that the moisture content of the samples was significantly different (p<0.05). In the meantime, the fourth treatment there was a significant difference. The fourth treatment the average moisture content varied with the average of other treatments and more (Table 2). *S. platensis* microalgae powder samples T2 and T1 moisture content was less than the control sample to other samples, the samples showed less moisture than the other. So it can be concluded that *S. platensis* microalgae could be a useful factor of the standard moisture content of the final product in order to avoid compromising the quality of the product.

**Effects on microbial properties**

Microbiological test results produced samples with control samples are presented in Table 3. That the microbial load no significant difference between samples containing *S. platensis* microalgae powder with a control sample (p>0.05).

There was no difference between the results of microbiological samples containing *S. platensis* microalgae powder with the national standards. So it can be concluded that *S. platensis* microalgae powder had a negative impact on microbial properties of the final product and the pasta is provided in accordance with standards (Table 3).

Table 3: microbiology test results pastes enriched with microalgae *Spirulina platensis*.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Limits (ISIRI., 2009)</th>
<th>T0</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total count of microorganisms (Cfu g&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>5 × 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>2 × 10&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Staphylococcus aureus (Cfu g&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Salmonella (25 gr)</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Clostridium perfringens (Cfu g&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Coliform (MPN g&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>10&lt;sup&gt;2&lt;/sup&gt;</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Mold count (Cfu g&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>10&lt;sup&gt;2&lt;/sup&gt;</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>&lt;10&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* N: Not detected

T0 (Treatment without microalgae powder (control)), T1 (Treatment with 0.25% microalgae powder), T2 (Treatment with 0.5% microalgae powder), T3 (Treatment with 0.75% microalgae powder), and T4 (Treatment with 1% microalgae powder)
**Sensory evaluation results**

Sensory evaluation test results on five indicators color, aroma, flavor, flavorful, texture and overall acceptability provided in Table 4. Based on the statistical results of the Index color, aroma and overall acceptability of the product, the first prototype (0.25%) to other treatments showed significant differences ($p<0.05$) and in terms of the three index points higher compared to other samples and control samples achieved. So sensory evaluation, the first sample containing *S. platensis* microalgae powder, T1 was selected as the best color (Fig. 1). Also the best flavor and the sweetest of the sensory evaluation, samples containing microalgae powder *S. platensis* is T1 (Fig. 2).

<table>
<thead>
<tr>
<th>Sensory characteristics</th>
<th>T0</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>2.550±1.096a</td>
<td>3.833±1.224a</td>
<td>2.800±1.086a</td>
<td>2.683±1.186a</td>
<td>2.583±1.344a</td>
</tr>
<tr>
<td>Odor</td>
<td>2.833±1.152a</td>
<td>3.567±1.419a</td>
<td>2.867±1.556a</td>
<td>2.967±1.327ab</td>
<td>2.733±1.339b</td>
</tr>
<tr>
<td>Taste</td>
<td>2.700±1.030a</td>
<td>3.750±1.159a</td>
<td>3.00±1.193a</td>
<td>2.883±1.415b</td>
<td>3.800±1.022b</td>
</tr>
<tr>
<td>Texture</td>
<td>2.733±1.219a</td>
<td>4.033±1.340a</td>
<td>3.133±1.512bc</td>
<td>2.900±1.231c</td>
<td>3.583±1.331ab</td>
</tr>
<tr>
<td>General acceptance</td>
<td>2.300±1.078a</td>
<td>4.217±0.865a</td>
<td>3.067±1.191a</td>
<td>3.133±0.965b</td>
<td>3.533±1.258b</td>
</tr>
</tbody>
</table>

* Values in the same line followed by different letters indicate significant differences ($p<0.05$).

The overall acceptability of the product, samples T1 the most beautiful specimens between samples and other control samples (Table 4) and Microalgae *S. platensis* powder on the entire sample T1 were general acceptance (Fig. 1).Thus, assessments, first and fourth samples were selected as the most delicious samples.

The index of tissue samples and the samples T4, T1 points higher, respectively (Fig. 1) and the first sample compared to other samples showed a significant difference ($p>0.05$). So in terms of sensory evaluation, the tissue sample containing *S. platensis* microalgae powder is capable of 0.25%. (Table 4).

**Figure 1: The results of sensory evaluation based on color rating.**

T0 (Treatment without microalgae powder (control)), T1 (Treatment with 0.25% microalgae powder), T2(Treatment with 0.5% microalgae powder), T3(Treatment with 0.75% microalgae powder), and T4(Treatment with 1% microalgae powder)
Discussion

Based on the research Salehifar et al. (2013), the amount of protein a significant increase compared to the control sample in muffins enriched with microalgae *S. platensis*. In general, chemical analysis of iron in the product range in the present study show that the average control the amount of iron compared to four treatments vary and iron content is less. The fourth treatment the highest average in terms of the amount of iron in the product compared to other treatments there. Salehifar et al. (2013) are also significant differences among the samples enriched with *Spirulina* and control in terms of the amount of iron expressed that according to the results of the study are present. These results are similar to results Mamatha et al. (2007) that they were able to prepare a snack of seaweed *Enteromorpha compressa*, Iron and calcium samples enriched with algae increase.

The fourth treatment means the amount of moisture with an average remaining different treatments and more. Overall, the present study shows that chemical analysis of the ash of the product range, averaging control the amount of ash in the desired product with other treatments mean there is a significant difference. Based on the research Lemes et al. (2012), the amount of ash in the samples treated with the control of *Spirulina* with no significant difference.

Overall, the study shows that chemical analysis of calcium in the product range, averaging control the amount of calcium in the product is significantly different from the mean other treatments.

This study shows that chemical analysis of phosphorus in the product range, averaging control the amount of phosphorus in the second and fourth product treatment means there is a significant difference.

Produced by microbial test results (Table 3) showed that microbial test different treatments such as control, in accordance with the Iranian National Standard No. 2393 and no problems in terms of microbial load and so on. According to the results Lemes et al. (2012), *Spirulina* products are manufactured according to the standard defined in terms of microbial load and no problem. In a study by Carlson (2011) was also pointed out that dozens of studies of human medicine so harmful effect of the supplement *Spirulina* is not provided. The Food and Drug Administration (FDA) GRAS term (generally recognized as safe and healthy) donated *Spirulina* prepared in two US companies. Similar to the results, the study Krishnakumari et al. (1981), toxicology studies effects of consuming *Spirulina* on humans and animals, including feeding up to 800 mg per kg and replacement of up to 60% of *Spirulina* protein, no toxic effect was presented. In general sensory evaluation results indicate that the subjects in the first treatment in terms of color, aroma and overall acceptability of the product is different than the four others. And the highest satisfaction and is friendly to the first treatment than others. The sensory evaluation showed that, in terms of the
subjects in the first and fourth treatment in terms of taste, is different than the other three and maximum satisfaction and friendly treatment than the rest of the first and fourth. Treated subjects in the first and fourth terms of texture is different than the other three and maximum satisfaction and friendly to the rest of the first treatment (Fig. 1).

The general acceptance of product features, samples containing S. platensis microalgae powder, 0.25%, as the most popular sessions and most of the sensory evaluation, received the most points (Fig. 1).

Fradique et al. (2010), enriched pasta with different values of the two microalgae Chlorella vulgaris and S. maxima did. In the study of microalgae can improve the quality indices enriched samples compared to control samples and color after cooking pasta microalgae remains relatively stable. On both microalgae, microalgae increasing concentrations (0.5 to 2 percent) increase was pasta firmness. In addition, Pasta with microalgae in sensory evaluation scores than the control sample had higher acceptance.

Overall, S. platensis microalgae powder samples have to have a green color, were accepted, which indicates that both the food and its nutritional properties, is important for the consumer. S. platensis microalgae powder samples has both features to their equipment. With increasing levels of consumer culture, people tend to use high nutritional value of pasta has been made.

In the end, the results of this study concluded that S. platensis microalgae powder can be evaluated in terms of features pasta improve the nutritional and sensory quality product can be produced that increase the nutritional value of pasta and is also real consumer satisfaction and friendly. It is suggested that to maintain the protein powder S. platensis microalgae in thermal process pasta, various heating methods used.

Acknowledgments
The authors wish to acknowledge the support of the Khoshnam food Group (MAK pasta) for their support of this work.

References


